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Flow cytometric analysis (FACS)

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An abbreviated version of this protocol was published in Science Advances in Jan 2021

Gut microbiota from patients with arteriosclerotic CSVD induces higher IL-17A production in neutrophils via activating RORγt

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Detailed protocol

A total of 2ml peripheral blood was obtained from patients or healthy controls with EDTA coated tube. After red blood cell lysis, cell suspensions were adjusted to a density of 1x106 cells in 100ul medium (RPMI1540 with 10%FBS) and stimulated with phorbol 12-myristate-13-acetate (PMA) and Inomycin with brefeldin A for 4h at 37°C. Cells were then stained with appropriate antibodies for surface markers (room temperature, 15min). The following antibodies were used for extracellular staining: CD66b (clone G10F5, 0.5ng/ul), CD14 (clone 63D3, 0.5ng/ul), CD3 (clone HIT3a, 0.5ng/ul), CD19 (clone 4G7, 0.5ng/ul), and CD11c (clone 3.9, 0.5ng/ul) from Biolegend. For intracellular staining, cells were fixed and permeabilized using Fixation and Permeabilization buffers from Invitrogen following the manufacturer's instructions. Cells were then stained with appropriated antibodies in Permeabilization Buffer overnight (4°C). The following antibodies were used: IL-17A (BL168, 1ng/ml). For FACS in mice splenocytes, cells were collected, stimulated and labelled with similar process detailed above. Mice neutrophils were not stimulated due to short half-life while the labeling process was similar. The following extracellular antibodies were used: Ly6G (clone 1A8, 0.5ng/ul), F4/80 (clone BM8, 0.5ng/ul), CD3 (clone 17A2, 0.5ng/ul), CD19 (clone 1D3/CD19, 0.5ng/ul) from Biolegend. The following intracellular antibodies were applied: IL-17A (clone eBio17B7, 1ng/ml), FoxP3 (clone FJK-16S, 1ng/ml), Perforin (clone eBioOMAK-D, 1ng/ml), Granzyme B (clone GB12, 1ng/ml), RORγt (clone AFKJS-9, 1ng/ml), phospho-STAT1-Ser727 (clone Stat1S727-C6, 1ng/ml) from Invitrogen (eBioscience). Isotype controls were used to establish compensation and gating parameters. Cells were then washed and analyzed with a flow cytometer (BD Biosciences) and the data were analyzed using the software of FlowJo 10.4 (with tSNE plugins). The tSNE analysis was performed automatically by the FlowJo software with indicated parameters

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Xiang, A. and Lu, Z. (2021). Flow cytometric analysis (FACS). Bio-protocol Preprint. bio-protocol.org/prep1023.
2. Cai, W., Chen, X., Men, X., Ruan, H., Hu, M., Liu, S., Lu, T., Liao, J., Zhang, B., Lu, D., Huang, Y., Fan, P., Rao, J., Lei, C., Wang, J., Ma, X., Zhu, Q., Li, L., Zhu, X., Hou, Y., Li, S., Dong, Q., Tian, Q., Ai, L., Luo, W., Zuo, M., Shen, L., Xie, C., Song, H., Xu, G., Zheng, K., Zhang, Z., Lu, Y., Qiu, W., Chen, T., Xiang, A. P. and Lu, Z.(2021). Gut microbiota from patients with arteriosclerotic CSVD induces higher IL-17A production in neutrophils via activating RORγt . Science Advances 7(4). DOI: 10.1126/sciadv.abe4827